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Award Number: W81XWH-09-2-0190

TITLE: “Natural History of Multi-Drug Resistant Organisms in a New Military Medical Facility”

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Tacoma, WA 98402

REPORT DATE: October 2012

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT:

X Approved for public release; distribution unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
October 01,2012	Annual	9/30/2011- 9/29/2012

4. TITLE AND SUBTITLE
"Natural History of Multi-Drug Resistant Organisms in a New Military Medical Facility"

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-09-2-0190

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

The Geneva Foundation
917 Pacific Ave, Suite 600
Tacoma, WA 98402

8. PERFORMING ORGANIZATION REPORT

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

A unique approach combines several platforms to assess BOTH the thoroughness AND the effectiveness of room cleaning in a newly constructed and opened hospital (FBCH). The former is accomplished using CDC developed protocols and an invisible dye- ultraviolet light system for sampling 17 high-touch surfaces (i.e. call buttons, bathroom hand rails, IV pumps/poles) before and after terminal room cleaning. This is incorporated into a web-based reporting and feedback system. Effectiveness of cleaning is accomplished using BOTH standard culture- based techniques AND molecular techniques - a real-time, species specific multiplex assay we have developed.

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18.NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			**19b. TELEPHONE NUMBER** *(include area code)*
U	U	U	UU	71	

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

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INTRODUCTION

The role of the environment in the transmission of important pathogens in the hospital is increasingly recognized. This study uses a unique approach that combines several platforms to assess BOTH the thoroughness AND the effectiveness of room cleaning in a newly constructed and opened hospital – Fort Belvoir Community Hospital (FBCH). The former is accomplished using CDC developed protocols and an invisible dye- ultraviolet light system for sampling 17 high-touch surfaces (i.e. call buttons, bathroom hand rails, IV pumps/poles) before and after terminal room cleaning. This is incorporated into a web-based reporting and feedback system. Effectiveness of cleaning is accomplished using BOTH standard culture- based techniques AND molecular techniques - a real-time, species specific multiplex assay we have developed. Results and feedback are relayed to staff in the form of educational sessions. Post intervention surveillance is then continued.

BODY

Major achievements to date include the garnering of protocol approval by the IRB, and a specific letter of support from the commander of FBCH, optimization of the molecular assay, use of the BROOM software to collect, barcode and store swab samples, and the collection of pre-opening baseline data, along with having collected 11 months of surveillance data that includes over 2500 samples from over 160 rooms in the new hospital.

Additionally, two sets of species-specific RT-PCR primers were designed for each of six clinically important bacteria, including *Acinetobacter baumannii, Clostridium difficile, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa*, and methicillin resistant *Staphylococcus aureus* (MRSA). Primer specificity and sensitivity was assessed using a panel of 42 bacterial species. Primers were subsequently used to detect the presence of bacteria from environmental and clinical swabs taken from both a primary and tertiary-care hospital. All swabs were directly treated with a rapid lysis buffer that facilitated maximum amplification. All twelve primer pairs were capable of detecting 10-50 gene copies with a high degree of specificity from pure bacterial cultures. The procedure was also capable of detecting bacterial contamination of clinical and laboratory work surfaces from pre-moistened swabs, comparable to results obtained from culture-based techniques. Subsequent analysis of the melting curves provided accurate identification of the contaminating species, both from control and test swabs. One manuscript is at press (Plos1) and another has been submitted.

In our original proposal and protocol we intended, and therefore budgeted only for, a nonspecific 16s bacterial DNA indicator. The success of the species specific assay led us to incorporate it into the surveillance. Reagents are significantly more expensive and more than 6x the originally anticipated volume of master mix is required. Additionally, the Dazo and Encompass systems and the 17-surface CDC protocol were not available when the original proposal was submitted in 2008. These enhancements have increased the projected cost of the study. We would also like to continue surveillance as long as possible after opening to reduce threats to validity such as seasonal fluctuations or asymmetric sample sizes- this means for a minimum of 12 months following the opening in October 2011.

KEY RESEARCH ACCOMPLISHMENTS

- Garnered protocol approval by the IRB, and a specific letter of support from the commander of Fort Belvior Community Hospital

- Optimized and integrated the molecular assay, with use of the BROOM software to collect, barcode and store swab samples, and with the Dazo and Encompass surveillance and reporting system

- Collected pre-opening baseline data in WRNMMC and FBCH

- Collected 11 months of surveillance data that includes over 2500 samples from over 160 rooms in the new hospital

- Delivered mid-point intervention in the form of feedback of results to the cleaning and infection control teams.

- Developed RT-PCR assay capable of detecting important nosocomial pathogens; (manuscript at press)

- Incorporation of Dazo/Encompass system provided important feedback about the thoroughness of cleaning to hospital staff – at no cost to the hospital. (Most hospitals pay for this because of its usefulness to the cleaning staff)

REPORTABLE OUTCOMES

All findings and outcomes relating to room cleanliness and contamination must be approved by the WRAIR and FBCH PAO before release or reporting because of their potentially sensitive nature. Some reportable outcomes- if permitted by PAO and OPSEC- will be:

1) The rates and trajectory of hospital contamination with multidrug-resistant organisms and *C. difficile.*
2) The percentages of surfaces cleaned before and after educational interventions
3) How cleaning correlated with rates of MDRO contamination
4) How molecular detection assays correlated with culture based assays.
5) If any MDRO isolated from the hospital surfaces were later isolated from patients wound or other infections

Potentially reportable findings for which we currently have data include:

- percentage of surfaces cleaned
- percentage of surfaces w/ culture-able bacterial before and after terminal cleaning
- percentage of surfaces w/ molecular evidence of bacteria before and after terminal cleaning
- if and how the above changed following the educational intervention
- concordance of culture results with molecular results
- type, amount, and location of multidrug-resistant organisms found

CONCLUSION

When finished, this study will have important- possibly landmark implications- for the role of standard of care and state of the art methods for assessing the thoroughness and effectiveness of cleaning in the hospital environment. It will be the first to have combined all three systems plus an educational intervention in a brand new hospital. Currently we are still collecting data in the 11th month in what is the planned minimum of at least 12 months of data collection following the hospital opening.

To date, very few MDRO have been isolated from the high-touch surfaces in the hospital. In December, we will stop collecting surveillance samples and begin the first major round of data analysis. At that time, a full report will follow.

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Clifford RJ, Milillo M, Prestwood J, Quintero R, Zurawski DV, Kwak YI, Waterman PE, Lesho EP, McGann P. Species-specific real-time PCR primers for the detection of clinical pathogens and bacterial 16S rRNA. Plos One. At press

APPENDICES

Species-specific real-time PCR primers for the detection of clinical pathogens and bacterial *16S* rRNA

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Running Title: Detection of clinical bacteria by real-time PCR

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Abstract

Within the paradigm of clinical infectious disease research, *Acinetobacter baumannii, Escherichia coli, Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* represent the four most clinically relevant, and hence most extensively studied bacteria. Current culture-based methods for identifying these organisms are slow and cumbersome, and there is increasing need for more rapid and accurate molecular detection methods.

Using bioinformatic tools, 962,279 bacterial *16S* rRNA gene sequences were aligned, and regions of homology were selected to generate a set of real-time PCR primers that target 93.6% of all bacterial 16S rRNA sequences published to date. A set of four species-specific real-time PCR primer pairs were also designed, capable of detecting less than 100 genome copies of *A. baumannii*, *E. coli*, *K. pneumoniae*, and *P. aeruginosa*. All primers were tested for specificity *in vitro* against 50 species of Gram-positive and –negative bacteria. Additionally, the species-specific primers were tested against a panel of 200 clinical isolates of each species, randomly selected from a large repository of clinical isolates from diverse areas and sources.

A comparison of culture and real-time PCR demonstrated 100% concordance. The primers were incorporated into a rapid assay capable of positive identification from plate or broth cultures in less than 90 minutes. Furthermore, our data demonstrate that current targets, such as the *uidA* gene in *E.coli*, are not suitable as species-specific genes due to sequence variation. The assay described herein is rapid, cost-effective and accurate, and can be easily incorporated into any research laboratory capable of real-time PCR.

Introduction

In clinical microbiology laboratories, traditional culture based techniques remain the primary methodology used for identifying bacterial isolates. These methods are time-consuming, can involve multiple biochemical tests, and can be expensive, particularly for fastidious organisms. In recent years, the introduction of automated identification instruments has resulted in greater reliability, but discrepancies between the various platforms have been reported [1-3]. Furthermore, many research laboratories lack the funds necessary to invest in these instruments, and often rely on minimal biochemical characterization or *16S* rRNA sequencing to confirm species type.

Despite significant advances in molecular biology, molecular methods for species identification have not achieved widespread use, with the possible exception of the Biodefense community [4]. Factors such as allelic variation within target genes, cross-reaction with other species [5] and the lack of experienced molecular technicians have all contributed to their relative scarcity. However, despite these limitations, molecular methods can provide significant advantages over phenotypic-based methods, including rapid turnaround time, scalability, and high sensitivity [5]. Furthermore, using appropriate bioinformatic tools and careful primer design, many of the limitations outlined above can be mitigated considerably.

Recent global calls [6-8] to help combat the spread of carbapenem-resistant *Enterobacteriaceae* (CRE), multi-drug resistant *A. baumannii* and *P.aeruginosa*, and methicillin-resistant *Staphylococcus aureus* (MRSA) has resulted in a plethora of research focused on these pathogens. A number of conventional PCR and real-time PCR assays have been developed targeting these organisms [9-13], but a comprehensive panel encompassing all four species is lacking.

We recently published a highly sensitive and specific real-time PCR assay for the detection of MRSA [14]. This report adds an additional four real-time PCR primers, designed using a combination of *in*

66 silico and *in* vitro methodologies that are highly specific to the four most common nosocomial
67 pathogens. We also describe a novel bacterial *16S* rRNA real-time PCR primer designed from the
68 alignment of 962,279 bacterial *16S r*RNA gene sequences, which will amplify a product from the *16S*
69 rRNA gene in 93.6% of all bacterial *16S* rRNA genes published to date.

Material and Methods

Primer Design

A complete list of the final primer panel and their primary characteristics is presented in Table 1. Unless noted otherwise, all primers were designed using Primer Express version 2.0 (Applied Biosystems, Carlsbad, CA). All primers were designed to have an optimal annealing temperature of 56^{o}C, and real-time PCR reactions were performed in 20 µl volumes with 200 nM primers. Where appropriate, alignment of individual gene sequences was performed using MegAlign version 10.0.1 (DNAStar Inc, Madison, WI).

16S real-time PCR primers

16S rRNA real-time PCR primers were designed manually from a consensus sequence based on an alignment of 962,279 bacterial *16S* rRNA gene sequences obtained from the Ribosomal Database Project release 10 [15]. To derive the consensus sequence, nucleotide frequencies were determined at each position in the alignment using a custom Perl script; positions where the majority of sequences had a gap were excluded. Regions of the consensus sequence where the majority nucleotide was present in >90% of the sequences were used for primer design. A synopsis of each nucleotide frequency is presented as supplemental material (Supplemental Table S1).

E. coli and *A. baumannii* – specific primers.

To identify genes specific to *E. coli* and *A. baumannii*, annotated complete genome sequences for 38 *E. coli* strains, and 8 completed and 52 draft *A. baumannii* genomes were selected (Supplemental Table S2 and S3). In addition, the two draft genomes of *A. nosocomialis* (RUH 2624 and NCTC 8102) and the four draft genomes of *A. pitii* (SH024, D499, DSM 21653, and DSM 9306) were used to ensure that all *Acinetobacter*-specific primers would also amplify targets from these two species also. For each protein in a strain, BLASTP [16] was used to find the best match to a protein family in the HOGENOM release

5 database [17]; orthologous proteins from different strains show the best match to the same HOGENOM protein family. Genes that are not single copy (that is, two or more proteins from the same strain are members of the same HOGENOM protein family) were excluded from further analysis. In addition genes for which the nucleotide length of any ortholog varies from the mean gene length by more than 5% were excluded in order to restrict the analysis to those genes that were highly conserved. For the genes that met these criteria and had the fewest variable positions, BLASTN analysis was performed against the NCBI non-redundant nucleotide database using a gene sequence randomly selected from the final species-specific gene targets.

Four genes showed no significant match to non-*Escherichia* species: HBG518163 (*hde*A), HBG636731 (Predicted secreted protein, function unknown, *ynfB*), HBG473849 (conserved protein, function unknown, *yccT*), and HBG758393 (Function unknown, DUF1418 family, *ybjC*). Nineteen genes showed no significant match to non-*Acinetobacter* species (Supplemental Table S4), and the top three candidates, based on total gene length and number of mismatches between all genomes; HBG594899 (*rpiN,* 50S ribosomal protein L14), HBG701403 (*secE,* preprotein translocase, SecE subunit), and HBG752450 (*scpB,* segregation and condensation protein B) were selected for further analysis.

Starting with a multiple sequence alignment, a custom Perl script was used to identify positions within each gene that are invariant among all strains. With *E. coli,* two of the four genes (*hdeA* and *yccA*) contained regions > 100bp that were suitable for primer design. All three genes from the *Acinetobacter* analysis contained extensively conserved regions suitable for primer design.

K. pneumoniae and _P. aeruginosa_ – specific primers

The limited number of published genome sequences available for *K. pneumoniae* and *P. aeruginosa* precluded extensive bioinformatic analysis. Therefore, the top two candidate genes from both species

(*K. pneumoniae*; *gltA* and *khe*; *P. aeruginosa; ecfX* and *23S*) were selected based on conserved gene sequences.

Validation of real-time PCR primers

All primer sets were tested for sensitivity, optimal annealing temperature and primer efficiency as previously described [18,19]. Primers were tested using two different instruments, the Roche Light Cycler 480 II (LC 480 II) with SYBR Green I Master Mix (Roche Applied Sciences, Indianapolis, IN), and the BioRad CFX96 with SsoAdvanced SYBR Green supermix (Bio-Rad Laboratories, Hercules, CA). Each primer was tested for specificity by two methods. First, the primers were tested against genomic DNA extracted from a panel of American Type Culture Collection (ATCC, Manassas, VA) and clinical isolates representing fifty different bacterial species, including closely related members from the same genus (Supplemental Table S5). Secondly, primers were tested against 200 clinical isolates of each species, identified to the species level using three automated identification systems; the Vitek 2 (bioMerieux, Durham, NC)), the BD Pheonix (Diagnostics Systems, Sparks, MD), and the Microscan Walkway (Siemens Healthcare Diagnostics Inc, Deerfield, IL), selected from a large repository of isolates (>10,000 strains) collected between 2002 to 2012 from 23 different facilities in the United States of America, Europe, Asia and the Middle East. Pulsed-field Gel Electrophoresis (PFGE) indicated that the selected clinical isolates represented a wide variety of different pulse-types (Unpublished results).

High throughput testing of clinical isolates

The primers were incorporated into a 96-well plate assay to allow high throughput testing of multiple clinical isolates. Single colonies of an overnight culture of each isolate were re-suspended in 200 µl of sterile, ultra-pure water and mixed by vortexing. 10 µl of the resulting suspension (or 10 µl taken directly from an overnight broth culture) was added to 20 µl of Lyse-and-go reagent (Thermo Scientific,

Waltham, MA) in 96-well plates, and run in a thermal cycler using the manufacturer's protocol for the isolation of total genomic DNA. Isolates were held at 80^{o}C for 15 minutes at the end of the program to maximize lysis, and 2 µl of the resulting lysate was used directly for real-time PCR. Leftover bacterial DNA in lyse-and-go reagent was stored at -20^{o}C, and no reduction in real-time PCR amplification was evidenced after 9 months (Data not shown). Appropriate positive (ATCC Type strains for each species), negative (Two ATCC type strains from species other than the target organism), and no template controls (Ultra-pure water) were incorporated onto every plate. Cycling parameters were 95^{o}C for 5 minutes, followed by 40 cycles of 95^{o}C for 10 seconds and 56^{o}C for 10 seconds. A melting curve analysis was included at the end of every program to assist in data analysis. Quantification cycle (Cq; CFX96) and crossing threshold (Ct; LC 480 II) values were calculated automatically using instrument software.

Results and Discussion

16S rRNA real-time PCR primers

Analysis of the alignment of 962,279 bacterial *16S* rRNA gene sequences revealed considerable nucleotide variation between species (Supplemental Table S1). However, two regions were identified that were suitable for primer design, where the nucleotide sequence was highly conserved in >90% of all rRNA sequences. Forward and Reverse primers were manually designed, with the nucleotide sequence of the forward primer being conserved in 98.5% of *16S* rRNA sequences, and the nucleotide sequence in the reverse primer being conserved in 93.8% (Supplemental Table S1).

Primers were tested against serial dilutions of genomic DNA from *A. bauamnnii* ATCC 19606, *E. coli* ATCC 35218, and *P. aeruginosa* ATCC 27853. The primers were reproducibly capable of detecting <100 copies of genomic DNA, and primer efficiency was 96.4%, 98.2% and 97.9% respectively, with an R^2 >99%. The primers successfully amplified a product from the 50 ATCC and clinical strains (Supplemental Table S5), providing *in vitro* support for the *in silico* primer design method employed.

The *16S* rRNA gene is a frequent target for many assays, and universal PCR primers are routinely used for species identification [20]. A number of real-time primers for this gene have also been developed, but they either contain degenerate nucleotides [21], or produce long amplicons that are not suitable for optimal real-time PCR [22]. The primer set described here overcomes both these limitations, and has proved a valuable tool in detecting bacterial contamination of environmental swabs (manuscript in preparation), and as a positive control for bacterial lysis.

Species-specific real-time PCR primers

Five sets of primers were tested for sensitivity and specificity for *A. baumannii;* three primers were generated using the genome alignment algorithm (*rpiN*, *secE* and *scpB*), one primer was designed in-house from an alignment of 54 *ompA* sequences, and the fifth primer, also targeting the *ompA* gene, has

been published previously [11]. *OmpA* was included as a potential target as this gene has been used as a target for *A. baumannii*-specific primers in previous publications [11]. However, though *ompA* was identified as a potential target using our algorithm, sequence variation among *A. baumannii*, *A. nosocomialis* and *A. pitii*, as well as variation in the protein length, resulted in this gene being flagged as unsuitable as an *Acinetobacter*-specific target. All five primers did not cross-react with other bacterial species, including *A. lwoffi* and *A. hemolyticus*. Primer efficiency at 56^{o}C, based on serial dilutions of *A. baumannii* AB0057 and *A. baumannii* ACICU, ranged from 97.2% (*rpiN*) to 99.4% (*secE*), with R^2 >99%, and all primers were reproducibly capable of detecting < 100 genome copies of DNA. Furthermore, all five primers successfully amplified products from the 200 clinical test isolates. The primer pair targeting the *secE* gene consistently provided greater sensitivity than all other primers at an annealing temperature of 56^{o}C, and was therefore selected as the optimal candidate for identifying *A. baumannii*. *In silico* analysis of the *secE* primer with the draft *A. nosocomialis* and *A. pitii* genomes demonstrated that these primers would also amplify a product from these two species.

Four sets of primers were tested for sensitivity and specificity for *E.coli*; two primers were generated using the genome alignment algorithm (*hdeA* and *yccT*), one primer was designed from an alignment of 38 *uidA* genes (Supplemental Table S2), and a fourth primer, also targeting the *uidA* gene, has been described previously [7,18]. *UidA* was included as this gene has been used as a species-specific target in a number of previous publications [10,12,23-25], but it did not meet the criteria demanded by the species-specific algorithm in this study. This was confirmed by both *in silico* and *in vitro* testing. An alignment of 38 *E.coli* genomes indicated that published *uidA* primer sequences [10,12] have 3 nucleotide variations in the forward primer and 1 nucleotide variation in the reverse primer when aligned with some *E.coli uidA* gene sequences. When tested against the 200 clinical isolates, the primers failed to amplify any product from 8 strains (Figure 1a). A second set of *uidA* primers was designed,

targeting a conserved region of the 38 *uidA* gene sequences. However, no amplification was again observed from 7 of the same 8 strains that were *uidA* negative from the first primer pair. One of these 7 isolates amplified a product after 28 cycles, indicative of inefficient primer annealing, most likely due to primer/target mismatches within the *uidA* gene.

The final two primers, targeting the *yccT* and *hdeA* genes, were identified as the most promising candidates based on the algorithm used in this study. Primer efficiency was 98.1% and 97.4% respectively, with R^2 >99%. However, 9 of the 200 clinical isolates failed to amplify a product with the *hdeA* primer, including 3 of the 8 *uidA*-negative isolates. In contrast, the *yccT* primer successfully amplified a product from all 200 isolates, and was therefore selected for as the final *E.coli*-specific primer set (Figure 1b). As expected, due to the very high homology between *E. coli* and *Shigella* strains [26], the yccT primer set also amplified a product from *Shigella flexneri* ATCC 12022, but did not cross-react with any other species.

Despite 38 sequenced genomes of *E. coli*, developing an *E. coli*-specific primer pair is challenging. We have shown that current targets, such as the *uidA* gene are not suitable due to considerably allelic variation, particularly among *E. coli* clinical isolates from diverse regions. The remarkable diversity among *E. coli* strains is also highlighted in the bioinformatic approach that we used, where just 2 candidate genes passed all of the criteria employed, and just a single gene, *yccT*, was eventually successful *in vitro*.

The paucity of completed genomic sequences for both *K. pneumoniae* and *P. aeruginosa* limited the application of our algorithm to just a single genome for *P .aeruginosa* with an additional 4 whole genome shotgun sequences, and 5 genomes for *K. pneumoniae*.

BLAST analysis suggested a potential cross-reaction between the *K. pneumoniae khe* primer set and *Citrobacter fruendii*, and this was confirmed *in vitro*. However, the *gltA* primer set demonstrated no

cross-reactivity with other species, a primer efficiency of 97.1%, and successfully amplified a product from all 200 clinical isolates of *K. pneumoniae*.

Both sets of *P. aeruginosa* primers demonstrated no cross-reactivity with other species, including *P. flourescens*, *P. stutzeri* and *P. putida*. However, when tested against a panel of 200 clinical isolates of *P. aeruginosa* only the *ecfX* primer successfully amplified a product from all strains, with 12 strains failing to generate a product with the *23S* rRNA primer set. The *ecfX* primer set demonstrated a primer efficiency of 93.8% with an R^2 value of 99.1%.

Species-specific primers targeting the *ecfX* gene in *P. aeruginosa* have been published for both conventional [27,28] and real-time PCR assays [9,29]. Our results support the continued use of this gene as a target for *P. aeruginosa*-specific primers, at least until further full genome sequences of this strain become available. In contrast, species-specific primers for *K. pneumoniae* are less well described, and primarily involve detecting targets that are specific to certain strains, such as the ST258 clone [30], or those with the hypermucoviscosity phenotype [31]. In addition, a number of conventional PCR assays to detect *K. pneumoniae* have been described [13,32], though to our knowledge, no assay described to date uses *gltA* as the target gene.

Conclusion

We describe a set of real-time PCR primers, designed to have the same optimal annealing temperature, and displaying high specificity for four clinically important pathogens. The primers are well suited for high-throughput testing of isolates, with results available in less than 90 minutes from bacterial colonies or overnight broth cultures. We also demonstrate the power of bioinformatics in designing optimal primer sequences, and provide a novel *16S* rRNA real-time PCR primer designed from the alignment of

239 over 960,000 bacterial *16S* rRNA sequences. The primers described herein have been an invaluable
240 addition to our surveillance network, and have demonstrated a 100% concordance with traditional
241 phenotypic identification systems.
242

Table 1 Primer characteristics

Name[1]	Gene	Target species	Sequence (5' to 3')	Eff (%)[3]	Length[4]
U16SRT-F	16S	variable	ACTCCTACGGGAGGCAGCAGT	>96.4%	140
U16SRT-F			TATTACCGCGGCTGCTGGC		
secERT-F		A.baumannii	GTTGTGGCTTTAGGTTTATTATACG		
secERT-R	secE	A. nosocomialis	AAGTTACTCGACGCAATTCG	99.4	94
secERT-Probe		A. pitii	ACCCATCAAGGTAAAGGCTTCGTTCG		
yccTRT-F		Escherichia coli	GCATCGTGACCACCTTGA		
yccTRT-R	yccT	Shigella spp.	CAGCGTGGTGGCAAAA	98.1	59
yccTRT-Probe			TGCATTATGTTTGCCGGTATCCG		
gltART-F	gltA	K. pneumoniae	ACGGCCGAATATGACGAATTC	97.1	68
gltART-R			AGAGTGATCTGCTCATGAA		
ecfXRT-F	ecfX	P.aeruginosa	AGCGTTCGTCCTGCACAAGT	93.8	81
ecfXRT-R			TCCACCATGCTCAGGGAGAT		

[1] Probe sequences were generated for the *secE* and *yccT* real-time PCR primers, but no *in vitro* testing was performed. The probe sequences are provided here for the benefit of researchers wishing to perform multiplex real-time PCR reactions using these primers. All primers have an optimal annealing temperature of 56^{o}C.

[2] Efficiency was calculated from the slope and intercept of the trendline produced following amplification with serial dilutions of genomic DNA from the ATCC strains of each species, as described previously [14].

[3] Amplicon size in base pairs

FIGURE LEGENDS

Figure 1. (A) Amplification curves of four *E. coli* isolates (MRSN 1628 – Red line, MRSN 1681 – Blue Line, MRSN 7544 – Green Line, and MRSN 7851 – Yellow Line) with *uidA* and *yccT* primers. MRSN 1628, 1681, and 7544 were negative for *uidA* but positive for *yccT*. MRSN 7851 was positive for both *uidA* and *yccT*. *E.coli* ATCC 35218 (Black line) was used as a positive control and *K. pneumoniae* ATCC 1706 (Grey Line) was used a negative control. Amplification curve is one sample from quintuplicate experiments. **(B)** Melting curve analysis of the amplicons produced by the *ycc* primer pair from 30 clinical isolates of *E. coli* demonstrating a highly conserved sequence in all strains. Melting curve of the *yccT* amplicon from *E. coli* ATCC 35218 is shown in red. All 30 isolates represented diverse pulse-types as determined by PFGE.

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SUPPORTING DATA

BELVOIR Bacteria

Bacteria	SampleGroup	RoomNumber	Surface
Shigella flexneri	Pediatric Ward	06.248	COMPOSITE 2
Pantoea agglomerans	Surgical Ward	06.342	Call box
Leclercia adecarboxylata	Surgical Ward	06.342	Bedside table
Pseudomonas putida	Surgical Ward	06.342	Bedside table
Pseudomonas luteola	Surgical Ward	06.342	COMPOSITE 1
Escherichia vulneris	Pediatric Ward	06.244	COMPOSITE 2
Pseudomonas putida	Pediatric Ward	06.244	COMPOSITE 2
Acinetobacter lwoffi	Pediatric Ward	06.244	COMPOSITE 1
Klebsiella pneumoniae ssp ozaenae	Pediatric Ward	06.244	COMPOSITE 1
Pantoea agglomerans	Pediatric Ward	06.244	Telephone
Pantoea agglomerans	Pediatric Ward	06.244	Toilet seat
Pantoea agglomerans	Pediatric Ward	06.244	Sink top
Shigella flexneri	Pediatric Ward	06.244	Sink top
Leclercia adecarboxylata	Pediatric Ward	06.244	Bedside table
Tatumella ptyseos	Pediatric Ward	06.248	Bathroom door closer
Enterobacter sakazakii	Pediatric Ward	06.248	COMPOSITE 2
Pantoea agglomerans	Medical Telemetry	07.334	Call box
Enterobacter sakzakii	Medical Telemetry	07.334	COMPOSITE 1
Pantoea agglomerans	Medical Telemetry	07.334	COMPOSITE 2
Pantoea agglomerans	Medical Telemetry	07.334	Sink top
Pseudomonas putida	Medical Telemetry	07.334	Sink top
Pantoea agglomerans	Medical Telemetry	07.334	Bathroom door closer
Pantoea agglomerans	Medical Telemetry	07.334	Telephone
Pantoea agglomerans	Medical Telemetry	07.334	Tray table
CDC group EO-2	Medical Telemetry	07.304	Room door closer
Enterobacter cloacae	Medical Telemetry	07.304	Sink top

Bacteria	SampleGroup	RoomNumber	Surface
Pseudomonas putida	Medical Telemetry	07.304	PT chair
Pantoea agglomerans	Medical Telemetry	07.328	Toilet rail
Shigella flexneri	Medical Telemetry	07.328	Toilet seat
Pantoea agglomerans	Medical Telemetry	07.328	Telephone
Pantoea agglomerans	Medical Telemetry	07.328	PT chair
Enterobacter sakazakii	Medical Telemetry	07.328	COMPOSITE 1
Pseudomonas oryzihabitans	Medical Telemetry	07.328	Bedpan cleaner
Shigella flexneri	Pediatric Ward	06.248	COMPOSITE 1
Rhizobium radiobacter	Medical Telemetry	07.334	Sink top
Staphyloccus saprophyticus	Medical Telemetry	07.334	Toilet rail
Enterobacter cloacae	Maternity Ward	05.312	COMPOSITE 1
Citrobacter koseri	Maternity Ward	05.312	Room door closer
Enterobacter cloacae	Maternity Ward	05.312	Sink top
Micrococcus luteus	Maternity Ward	05.312	Telephone
Pantoea agglomerans	Maternity Ward	05.312	Toilet seat
Enterobacter cloacae	Maternity Ward	05.312	COMPOSITE 2
Stenotrophomonas maltophilia	Maternity Ward	05.312	COMPOSITE 2
Pseudomonas putida	Maternity Ward	05.312	Bedside table
Stenotrophomonas maltophilia	Pediatric Ward	06.244	Sink top
Pantoea agglomerans	Pediatric Ward	06.244	COMPOSITE 2
Acinetobacter lwoffi/haemolyticus	ICU	02.122	Call box
Staphylococcus saprophyticus	ICU	02.123	COMPOSITE 2
Pantoea agglomerans	ICU	02.123	Bedside table
Staphyloccus saprophyticus	ICU	02.123	Tray table
Staphyloccus saprophyticus	Surgical Ward	06.342	PT chair
Pseudomonas putida	Surgical Ward	06.342	Sink top
Aerococcous viridans	Surgical Ward	06.342	Call box
Rhizobium radiobacter	Surgical Ward	06.342	Bedside table
Staphyloccus warneri	Surgical Ward	06.342	Telephone
Staphylococcus saprophyticus	Surgical Ward	06.342	COMPOSITE 2
Aerococcus viridans	Surgical Ward	06.342	Tray table

Bacteria	SampleGroup	RoomNumber	Surface
Pseudomonas putida	Surgical Ward	06.342	COMPOSITE 1
Enterobacter cloacae	Maternity Ward	05.312	COMPOSITE 1
Pseudomonas putida	Maternity Ward	05.312	COMPOSITE 1
Pseudomonas putida	Maternity Ward	05.312	Sink top
Pseudomonas putida	Maternity Ward	05.312	PT chair
Pseudomonas putida	Maternity Ward	05.312	Tray table
Acinetobacter lwoffii	Maternity Ward	05.312	Bedside table
Acinetobacter lwoffii/haemolyticus	Maternity Ward	05.312	Telephone
Escherichia coli	Maternity Ward	05.312	Toilet seat
Enterobacter cloacae	Maternity Ward	05.312	COMPOSITE 2
Pseudomonas putida	Maternity Ward	05.312	Toilet handle
Moraxella species	Surgical Ward	06.338	Sink top
Pseudomonas putida	Surgical Ward	06.338	Telephone
Delftia acidovorans	Medical Telemetry	07.328	COMPOSITE 1
Staphyloccus saprophyticus	Medical Telemetry	07.328	Toilet rail
Escherichia coli	ICU	02.122	Toilet seat
Acinetobacter baumannii/calcoaceticus complex	ICU	02.122	Call box
Acinetobacter lwoffii	ICU	02.122	Tray table
Citrobacter freundii	Surgical Ward	06.338	Toilet rail
Klebsiella pneumoniae ssp ozaenae	Surgical Ward	06.338	COMPOSITE 1
Pantoea agglomerans	Medical Telemetry	07.304	COMPOSITE 2
Pantoea agglomerans	Medical Telemetry	07.304	Sink top
Pantoea agglomerans	Medical Telemetry	07.304	COMPOSITE 1
Staphyloccus saprophyticus	Medical Telemetry	07.304	Toilet seat
Acinetobacter baumannii/calcoaceticus complex	Medical Telemetry	07.342	Toilet handle
Acinetobacter baumannii/calcoaceticus complex	Medical Telemetry	07.342	Sink top
Acinetobacter baumannii/calcoaceticus complex	Medical Telemetry	07.342	Toilet seat
Acinetobacter baumannii	Medical Telemetry	07.342	Call box
Acinetobacter baumannii/calcoaceticus complex	Medical Telemetry	07.342	Toilet rail

Bacteria	SampleGroup	RoomNumber	Surface
Acinetobacter baumannii	Medical Telemetry	07.342	Bathroom door closer
Acinetobacter baumannii/calcoaceticus complex	Medical Telemetry	07.342	Telephone
Acinetobacter baumannii	Medical Telemetry	07.342	Room Sink
CDC Group EF-4b	Medical Telemetry	07.342	Chair
Escherichia coli	Medical Ward	07.244	Toilet seat
Acinetobacter lwoffii/haemolyticus	Surgical Ward	06.316	Tray table
Acinetobacter baumannii	Surgical Ward	06.316	Room Sink
Acinetobacter lwoffii/haemolyticus	Surgical Ward	06.316	Bedpan cleaner
Serratia marcescens	Maternity Ward	05.322	Sink top
Acinetobacter lwoffii	Maternity Ward	05.322	Bedside table
Ochrobactrum anthropi	Maternity Ward	05.322	Chair
Acinetobacter baumannii/cacoaceticus complex	Surgical Ward	06.316	Toilet rail
Acinetobacter baumannii	Surgical Ward	06.316	IV Pole
Pseudomonas putida	Surgical Ward	06.316	Bedside table
Pseudomonas luteola	Surgical Ward	06.316	Chair
Acinetobacter baumannii/cacoaceticus complex	Surgical Ward	06.316	Tray table
Acinetobacter baumannii/calcoaceticus complex	Surgical Ward	06.316	Room Sink
Acinetobacter species	Surgical Ward	06.316	Room Sink
Staphylococcus schleiferi ssp coagulans	Surgical Ward	06.316	Room Sink
Acinetobacter baumannii	Surgical Ward	06.316	Toilet seat
Acinetobacter baumannii	Surgical Ward	06.316	Call box
Acinetobacter baumannii/calcoaceticus complex	Surgical Ward	06.316	Sink top
Micrococcus luteus	Medical Ward	07.216	Sink top
Micrococcus luteus	Medical Ward	07.216	Room Sink
Staphylococcus sciuri	ICU	02.123	Toilet seat
Staphylococcus sciuri	ICU	02.123	Toilet rail
Aerococcus viridans	ICU	02.123	Toilet handle
Staphylococcus sciuri	ICU	02.123	Toilet handle
Enterococcus casseliflavus/gallinarum	ICU	02.123	Bedside table
Micrococcus luteus	ICU	02.123	Telephone

Bacteria	SampleGroup	RoomNumber	Surface
Staphylococcus saprophyticus	ICU	02.123	Chair
Aerococcus viridans	ICU	02.123	Tray table
Pantoea agglomerans	ICU	02.123	Room Sink
Micrococcus luteus	ICU	02.123	Room door closer
Citrobacter koseri	ICU	02.123	Sink top
Klebsiella pneumoniae ssp pneumoniae	ICU	02.123	Sink top
Moraxella species	Medical Ward	07.242	Sink top
Klebsiella pneumoniae ssp pneumoniae	Medical Ward	07.242	Chair
Acinetobacter lwoffii/haemolyticus	Surgical Ward	06.312	Toilet rail
Micrococcus lylae	Surgical Ward	06.312	IV Pole
Pseudomonas aeruginosa	Maternity Ward	05.334	Sink top
Stenotrophomonas maltophilia	Maternity Ward	05.334	Chair
Escherichia coli	Maternity Ward	05.334	Toilet seat
Pantoea agglomerans	Maternity Ward	05.334	Toilet seat
Micrococcus luteus	Maternity Ward	05.334	Room door closer
Pantoea agglomerans	Medical Telemetry	07.336	Room Sink
Shigella dysenteriae	Medical Telemetry	07.336	Room Sink
Micrococcus luteus	Medical Telemetry	07.336	Call box
Micrococcus luteus	Medical Telemetry	07.336	Toilet seat
Micrococcus luteus	Medical Telemetry	07.336	Bathroom door closer
Acinetobacter lwoffii	Medical Telemetry	07.336	Tray table
Pantoea agglomerans	Medical Telemetry	07.336	Tray table
Leclercia adecarboxylata	Medical Telemetry	07.336	Chair
Sphingomonas paucimobilis	Medical Telemetry	07.336	Room Sink
Micrococcus luteus	Medical Ward	07.216	Room door closer
Micrococcus luteus	ICU	02.123	Toilet seat
Pseudomonas putida	ICU	02.123	Room Sink

Bacteria	SampleGroup	RoomNumber	Surface
Acinetobacter lwoffii	Maternity Ward	05.334	Toilet rail
Pseudomonas putida	Maternity Ward	05.334	Sink top
Pantoea agglomerans	Surgical Ward	06.312	Bathroom door closer
Pantoea agglomerans	Surgical Ward	06.312	Chair
Micrococcus luteus	Surgical Ward	06.312	Bedside table
Staphylococcus haemolyticus	ICU	02.114	Sink top
Staphylococcus hominis	ICU	02.114	Chair
Staphylococcus epidermis	ICU	02.114	Room Lightswitch
Staphylococcus hominis	ICU	02.114	Toilet handle
Staphylococcus hominis	ICU	02.114	Bathroom door closer
Enterococcus faecium	ICU	02.116	Toilet handle
Enterococcus faecium	ICU	02.116	Toilet handle
Staphylococcus hominis	ICU	02.116	Call box
Staphylococcus epidermis	ICU	02.116	Bedside table
Staphylococcus capitis	ICU	02.114	Tray Table
Staphylococcus haemolyticus	ICU	02.114	Chair
Staphylococcus haemolyticus	ICU	02.116	Bedside table
Staphylococcus aureus	Pediatric Ward	06.218	Side rail
Staphylococcus epidermis	Pediatric Ward	06.218	Bedside table
Staphylococcus epidermis	Pediatric Ward	06.218	Room Chair
Staphylococcus hominis	Pediatric Ward	06.218	Room Sink
Staphylococcus simulans	Pediatric Ward	06.218	Sink top
Staphylococcus simulans	Pediatric Ward	06.218	Toilet seat
Staphylococcus epidermis	Pediatric Ward	06.218	Room door closer
Staphylococcus haemolyticus	Maternity Ward	05.330	Toilet rail
Staphylococcus aureus	Maternity Ward	05.330	Sink top
Staphylococcus haemolyticus	Maternity Ward	05.314	Toilet rail
Klebsiella oxytoca	Maternity Ward	05.314	Sink top
Staphylococcus haemolyticus	Maternity Ward	05.314	Sink top
Staphylococcus warneri	Maternity Ward	05.314	Tray table
Staphylococcus capitis ssp ureolyticus	Maternity Ward	05.314	Room door closer
Staphylococcus epidermis	Maternity Ward	05.314	Bathroom door closer
Staphylococcus warneri	Maternity Ward	05.314	Bathroom door closer
Staphylococcus haemolyticus	Maternity Ward	05.314	Bedside table

Bacteria	SampleGroup	RoomNumber	Surface
Staphylococcus haemolyticus	Maternity Ward	05.314	IV Pole
Staphylococcus haemolyticus	Maternity Ward	05.314	Room Chair
Staphylococcus haemolyticus	Medical Telemetry	07.314	Telephone
Staphylococcus haemolyticus	Medical Telemetry	07.314	Room Chair
Staphylococcus haemolyticus	Medical Telemetry	07.314	Tray table
Staphylococcus haemolyticus	Medical Telemetry	07.314	Sink top
Staphylococcus haemolyticus	Medical Telemetry	07.314	Bedpan cleaner
Staphylococcus cohnii ssp cohnii	Medical Telemetry	07.314	Room Sink
Staphylococcus haemolyticus	Medical Telemetry	07.314	Toilet seat
Staphylococcus simulans	Medical Telemetry	07.314	IV Pole
Staphylococcus haemolyticus	Medical Telemetry	07.314	Bedside table
Staphylococcus haemolyticus	Medical Telemetry	07.334	Room Sink
Staphylococcus epidermis	Medical Telemetry	07.334	Toilet seat
Staphylococcus haemolyticus	Medical Telemetry	07.334	Toilet rail
Staphylococcus acidominimus	Medical Telemetry	07.334	Room door closer
Staphylococcus aureus	Medical Telemetry	07.334	Telephone
Staphylococcus aureus	Medical Ward	07.230	Side rail
Staphylococcus hominis	Medical Ward	07.230	Room Sink
Micrococcus luteus	Medical Telemetry	07.314	Bathroom lightswitch
Stenotrophomonas maltophilia	Medical Telemetry	07.314	Sink top
Acinetobacter baumannii/calcoaceticus complex	Medical Telemetry	07.334	Sink top
Micrococcus luteus	Medical Telemetry	07.334	Bedside table

Bacteria	SampleGroup	RoomNumber	Surface
Staphylococcus saprophyticus	Medical Telemetry	07.334	Bedside table
Staphylococcus aureus	Medical Telemetry	07.334	Call box
Micrococcus lylae	Medical Telemetry	07.334	Toilet seat
Staphylococcus aureus	Medical Telemetry	07.334	Toilet rail
Klebsiella oxytoca	Pediatric Ward	06.218	Tray table
Leclercia adecarboxylata	Pediatric Ward	06.218	Tray table
Serratia plymuthica	Pediatric Ward	06.218	Tray table
Escherichia vulneris	Pediatric Ward	06.218	Sink top
Acinetobacter baumannii/calcoaceticus complex	Pediatric Ward	06.218	Room Sink
Pseudomonas putida	Pediatric Ward	06.218	Room Sink
Micrococcus lylae	Pediatric Ward	06.218	Toilet seat
Staphylococcus capitis	Maternity Ward	05.314	Call box
Micrococcus luteus	Maternity Ward	05.314	Bedside table
Staphyococcus saprophyticus	Maternity Ward	05.314	Bedside table
Staphylococcus epidermidis	Maternity Ward	05.314	Bedpan cleaner
Pseudomonas putida	Maternity Ward	05.314	Sink top
Staphylococcus saprophyticus	Maternity Ward	05.314	Telephone
Pseudomonas fluorescens	Maternity Ward	05.330	Room Chair
Staphyococcus saprophyticus	Maternity Ward	05.330	Bedside table
Pseudomonas orzihabitans	Medical Ward	07.208	Room Chair
Klebsiella pneumoniae ssp ozaenae	Medical Ward	07.208	Sink top
Pseudomonas putida	Medical Ward	07.208	Sink top
Serratia liquefaciens	Medical Ward	07.208	Sink top
Staphylococcus warneri	Medical Ward	07.230	Toilet rail
Acinetobacter baumannii/calcoaceticus complex	Medical Ward	07.230	Sink top
Pseudomonas fluorescens	Medical Ward	07.230	Sink top
Enterococcus faecium	Medical Ward	07.230	Bedpan cleaner
Acinetobacter baumannii/cacloaceticus complex	Medical Ward	07.230	Room Sink
Pseudomonas putida	Pediatric Ward	06.216	Sink top
Acinetobacter baumannii	Pediatric Ward	06.218	Sink top
Enterobacter cloacae	Maternity Ward	05.340	Sink top
Pseudomonas putida	Maternity Ward	05.340	Sink top

Bacteria	SampleGroup	RoomNumber	Surface
Micrococcus lylae	Pediatric Ward	06.212	Side rail
Acinetobacter baumannii/calcoaceticus complex	Pediatric Ward	06.212	Toilet rail
Staphylococcus aureus	Surgical Ward	06.316	Room Chair
Pseudomonas aeruginosa	Surgical Ward	06.316	Sink top
Serratia marcescens	ICU	02.123	Tray table
Enterobacter cloacae	ICU	02.123	Toilet seat
Escherichia coli	ICU	02.123	Toilet seat
Klebsiella oxytoca	ICU	02.123	Toilet seat
Citrobacter werkmanii	ICU	02.123	Sink top
Moraxella species	ICU	02.123	Bathroom door closer
Pangoea agglomerans	ICU	02.123	IV Pole
Pantoea agglomerans	ICU	02.123	IV Pole
Pantoea agglomerans	ICU	02.123	Tray table
Serratia odorifera 1	Medical Ward	07.234	Sink top
Moraxella species	Medical Telemetry	07.314	Telephone
Aerococcus viridans	Medical Ward	07.236	Toilet rail
Enterobacter aerogenes	Medical Ward	07.236	Sink top
Escherichia coli	Medical Ward	07.236	Sink top
Staphylococcus aureus	Medical Ward	07.236	Sink top
Staphylococcus aureus	Medical Ward	07.236	IV Pole
Staphylococcus aureus	Medical Ward	07.236	Bathroom lightswitch
Acinetobacter lwoffii	Pediatric Ward	06.212	Bathroom door closer
Pantoea agglomerans	Pediatric Ward	06.212	Telephone
Pantoea agglomerans	Surgical Ward	06.314	Sink top
Klebsiella pneumoniae	Maternity Ward	05.332	Sink top
Acinetobacter lwoffii	Maternity Ward	05.338	Sink top
Acinetobacter lwoffii	Maternity Ward	05.338	Room Chair
Stenotrophomonas maltophilia	Maternity Ward	05.338	Toilet rail
Acinetobacter lwoffii	Maternity Ward	05.338	Bedpan cleaner
Acinetobacter lwoffii	Maternity Ward	05.338	Bedside table
Acinetobacter lwoffii	Maternity Ward	05.338	Toilet seat
Staphylococcus hyicus	Surgical Ward	06.314	Call box
Arcanobacterium pyogenes	Surgical Ward	06.314	IV Pole
Arcanobacterium pyogenes	Surgical Ward	06.314	Bathroom door closer

Bacteria	SampleGroup	RoomNumber	Surface
Arcanobacterium pyogenes	Surgical Ward	06.314	Toilet rail
Pseudomonas oryzihabitans	Medical Telemetry	07.314	Toilet seat
Staphylococcus haemolyticus	Medical Telemetry	07.314	IV Pole
Acinetobacter lwoffii	Maternity Ward	05.338	Toilet seat
Staphylococcus hominis	Maternity Ward	05.338	IV Pole
Pantoea agglomerans	Maternity Ward	05.338	Toilet rail
Acinetobacter lwoffii	Maternity Ward	05.338	Room Chair
CDC group EO-2	Maternity Ward	05.338	Telephone
Staphylococcus lugdunesis	Medical Ward	07.236	Bedside table
Enterobacter cloacae	Medical Ward	07.236	Sink top
Klebsiella oxytoca	Medical Ward	07.236	Sink top
Suttonella indologenes	Medical Ward	07.236	Sink top
Pantoea agglomerans	Medical Ward	07.236	Bathroom lightswitch
Staphylococcus epidermidis	Maternity Ward	05.332	Bedside table
Staphylococcus hominis	Maternity Ward	05.332	Telephone
Pantoea agglomerans	Maternity Ward	05.332	Tray table
Pseudomonas fluorescens	Maternity Ward	05.332	Toilet handle
Staphylococcus epidermidis	Maternity Ward	05.332	Sink top
Pseudomonas fluorescens	Pediatric Ward	06.212	Room Lightswitch
Pantoea agglomerans	Pediatric Ward	06.212	Toilet rail
Staphylococcus haemolyticus	Maternity Ward	05.338	Bedside table
Dermacoccus nishinomiyaensis	Maternity Ward	05.338	Telephone
Staphylococcus capitis ssp ureolyticus	Maternity Ward	05.338	Telephone
Staphylococcus haemolyticus	Maternity Ward	05.338	Tray table
Enterobacter cloacae	Maternity Ward	05.338	Room Sink
Staphylococcus haemolyticus	Maternity Ward	05.338	IV Pole
Staphylococcus epidermidis	Maternity Ward	05.338	Toilet seat
Staphylococcus haemolyticus	Maternity Ward	05.338	Toilet rail
Enterobacter cloacae	Maternity Ward	05.338	Sink top
Pseudomonas putida	Maternity Ward	05.338	Sink top
Stenotrophomonas maltophilia	Surgical Ward	06.344	Toilet rail
Staphylococcus hominis	Surgical Ward	06.344	Telephone
Staphylococcus epidermidis	Surgical Ward	06.344	Bedside table
Staphylococcus epidermidis	Medical Telemetry	07.328	Toilet seat

Bacteria	SampleGroup	RoomNumber	Surface
Staphylococcus equorum	Medical Telemetry	07.328	Bedside table
Staphylococcus haemolyticus	Medical Telemetry	07.328	Toilet rail
Pseudomonas putida	Medical Telemetry	07.328	Sink top
Acinetobacter baumannii/calcoaceticus complex	Medical Telemetry	07.328	Room Lightswitch
Acinetobacter baumannii/calcoaceticus complex	Medical Telemetry	07.328	Room Chair
Staphylococcus haemolyticus	Medical Telemetry	07.328	Room Chair
Staphylococcus haemolyticus	Medical Telemetry	07.328	Call box
Pseudomonas fluorescens	Medical Telemetry	07.328	Tray table
Klebsiella pneumoniae ssp pneumoniae	Medical Telemetry	07.328	Telephone
Acinetobacter baumannii/calcoaceticus complex	Medical Telemetry	07.328	Side rail
Staphylococcus haemolyticus	Medical Telemetry	07.328	Room Sink
Staphylococcus haemolyticus	Maternity Ward	05.336	Bathroom door closer
Staphylococcus haemolyticus	Maternity Ward	05.336	Tray table
Staphylococcus haemolyticus	Maternity Ward	05.336	Toilet rail
Staphylococcus haemolyticus	Maternity Ward	05.336	Toilet handle
Klebsiella oxytoca	Maternity Ward	05.336	Sink top
Pseudomonas putida	Maternity Ward	05.336	Sink top
Staphylococcus haemolyticus	Maternity Ward	05.336	Toilet seat
Staphylococcus simulans	Surgical Ward	06.344	IV Pole
Streptococcus acidominimus	Surgical Ward	06.344	Sink top
Micrococcus luteus	Surgical Ward	06.344	Call box
Micrococcus luteus	Maternity Ward	05.336	Telephone
Staphylococcus haemolyticus	Maternity Ward	05.336	IV Pole
Pantoea agglomerans	Maternity Ward	05.336	Toilet rail
Pseudomonas oryzihabitans	Maternity Ward	05.336	Tray table
Moraxella (Branhamella) catarrhalis	Maternity Ward	05.338	Room Chair
Acinetobacter lwoffii	Maternity Ward	05.338	Toilet rail
Pseudomonas luteola	Maternity Ward	05.338	Telephone
Dermacoccus nishinomiyaensis	Maternity Ward	05.338	IV Pole

Bacteria	SampleGroup	RoomNumber	Surface
Enterobacter cloacae	Maternity Ward	05.338	Room Sink
Esherichia vulneris	Maternity Ward	05.338	Room Sink
Acinetobacter species	Maternity Ward	05.338	Sink top
Aerococcus viridans	Medical Ward	07.236	Tray table
Staphylococcus saprophyticus	ICU	02.121	Telephone
Bacillus megaterium	Maternity Ward	05.326	Call box
Pantoea agglomerans	Maternity Ward	05.326	Toilet rail
Staphylococcus aureus	Maternity Ward	05.326	Toilet rail
Staphylococcus haemolyticus	ICU	02.121	Bedside table
Staphylococcus aureus	ICU	02.121	Sink top
Staphylococcus saprophyticus	ICU	02.121	Room Chair
Staphylococcus haemolyticus	ICU	02.121	Toilet rail
Staphylococcus epidermidis	ICU	02.121	Room Sink
Staphylococcus saprophyticus	ICU	02.121	IV Pole
Pseudomonas species	Maternity Ward	05.326	Room Sink
Staphylococcus hominis	Maternity Ward	05.326	Bedside table
Citrobacter freundii	Maternity Ward	05.326	Sink top
Pseudomonas aeruginosa	Maternity Ward	05.326	Sink top
Pseudomonas putida	Maternity Ward	05.326	Sink top
Staphylococcus haemolyticus	Maternity Ward	05.326	Toilet rail
Staphylococcus haemolyticus	Medical Telemetry	07.332	Room Sink
Staphylococcus hominis	Medical Telemetry	07.332	Bathroom lightswitch
Staphylococcus hominis	Medical Telemetry	07.332	Bedside table
Staphylococcus haemolyticus	Medical Telemetry	07.332	IV Pole
Staphylococcus capitis	Medical Telemetry	07.332	Toilet rail
Staphylococcus hominis	Medical Telemetry	07.332	Toilet seat
Staphylococcus haemolyticus	Medical Telemetry	07.332	Sink top
Corynebacterium amycolatum/minutissimum	Medical Ward	07.236	Sink top
Aerococcus viridans	Medical Ward	07.236	Room Sink
Acinetobacter lwoffii	Medical Ward	07.236	Tray table
Staphylococcu haemolyticus	Medical Telemetry	07.332	Toilet seat

Bacteria	SampleGroup	RoomNumber	Surface
Aerococcus viridans	Medical Telemetry	07.342	Toilet handle
Staphylococcus epidermidis	Medical Telemetry	07.342	Toilet seat
Staphylococcus haemolyticus	Medical Telemetry	07.342	Sink top
Aerococcus viridans	Medical Telemetry	07.342	IV Pole
Staphylococcus hominis	Medical Telemetry	07.342	Toilet rail
Staphylococcus capitis ssp capitis	Medical Ward	07.242	Room Sink
Enterobacter aerogenes	Medical Ward	07.242	Toilet rail
Enterobacter aerogenes	Medical Ward	07.242	Toilet handle
Pseudomonas aeruginosa	Medical Ward	07.242	Sink top
Staphylococcus epidermidis	Medical Ward	07.242	Tray table
Staphylococcus capitis	Medical Ward	07.242	Bathroom door closer
Staphylococcus gallinarum	Medical Ward	07.242	Room Chair
Enterobacter aerogenes	Medical Ward	07.242	Toilet seat
Escherichia coli	Medical Ward	07.242	Toilet seat
Staphylococcus aureus	Medical Ward	07.242	Toilet seat
Aerococcus viridans	Surgical Ward	06.344	Side rail
Staphylococcus epidermidis	Surgical Ward	06.344	Bedside table
Staphylococcus capitis ssp ureolyticus	Surgical Ward	06.344	Sink top
Pantoea agglomerans	Surgical Ward	06.344	Room Chair
Staphylococcus simulans	Surgical Ward	06.344	Room Chair
Corynebacterium urealyticum	Surgical Ward	06.344	Telephone
Pantoea agglomerans	Surgical Ward	06.344	Toilet rail
Pseudomonas putida	Surgical Ward	06.344	Toilet rail
Staphylococcus carnosus	Surgical Ward	06.344	Toilet rail
Alcaligenes faecalis	Maternity Ward	05.336	Bedside table
Dermacoccus nishinomiyaensis	Maternity Ward	05.336	Bedside table
Kocuria varians	Maternity Ward	05.336	Room Chair
Moraxella species	Maternity Ward	05.336	Tray table
Kytococcus sedentarius	Maternity Ward	05.336	Telephone
Acinetobacter baumannii	Maternity Ward	05.336	Toilet rail
Kocuria varians	Maternity Ward	05.336	Toilet rail
Acinetobacter lwoffii/haemolyticus	Maternity Ward	05.336	Toilet seat
Acinetobacter lwoffii	Maternity Ward	05.336	Room Sink
Alcaligenes faecalis	Maternity Ward	05.336	IV Pole

Bacteria	SampleGroup	RoomNumber	Surface
Leclercia adecarboxylata	Maternity Ward	05.336	Call box
Pseudomonas putida	Maternity Ward	05.336	Call box
Klebsiella oxytoca	Maternity Ward	05.336	Sink top
Oligella urethralis	Maternity Ward	05.336	Sink top
Kocuria rosea	Medical Ward	07.242	Room Chair
Staphylococcus haemolyticus	Medical Ward	07.242	Bedside table
Enterobacter cloacae	Medical Ward	07.242	Room Sink
Staphylococcus hominis	Medical Ward	07.242	Toilet rail
Staphylococcus warneri	Medical Ward	07.242	Toilet seat
Enterococcus faecalis	Medical Ward	07.242	Tray table
Staphylococcus hominis	Medical Telemetry	07.342	Toilet rail
Staphylococcus warneri	Maternity Ward	05.336	Room Sink
Staphylococcus haemolyticus	Maternity Ward	05.336	Call box
Staphylococcus epidermidis	Maternity Ward	05.336	Telephone
Staphylococcus capitis	Maternity Ward	05.336	Toilet handle
Pseudomonas putida	Maternity Ward	05.336	Bathroom lightswitch
Staphylococcus chromogenes	Maternity Ward	05.336	Bathroom door closer
Staphylococcus hominis	Maternity Ward	05.336	Room Chair
Staphylococcus warneri	Maternity Ward	05.336	Bedpan cleaner
Citrobacter amalonaticus	Maternity Ward	05.336	Sink top
Klebsiella oxytoca	Maternity Ward	05.336	Sink top
Pseudomonas putida	Maternity Ward	05.336	Sink top
Acinetobacter lwoffii	Maternity Ward	05.336	Toilet rail
Kocuria varians	Maternity Ward	05.336	Toilet rail
Staphylococcus epidermidis	Maternity Ward	05.336	Room door closer

BELVOIR Molecular Before-After Comparison

RoomNumber	SampleGroup	1-SampleID	1-16S	2-SampleID	2-16S
02.116	ICU	1238	+	1268	-
02.122	ICU	661	+	750	+
02.122	ICU	662	+	760	-
02.122	ICU	660	-	753	+
02.122	ICU	658	+		
02.122	ICU	657	+	754	+
02.122	ICU	656	+	762	+
02.122	ICU	654	+	761	-
02.122	ICU	650	+	759	+
02.123	ICU	670	+		
02.123	ICU	1026	+	1165	+
02.123	ICU	1031	+	1169	+
02.123	ICU	1029	+	1163	+
02.123	ICU	1028	+	1159	-
02.123	ICU	1027	-	1170	+
02.123	ICU	1032	+	1166	+
02.123	ICU	1024	+	1164	+
02.123	ICU	1021	+	1168	+
02.123	ICU	1034	+	1156	+
02.123	ICU	675	+		
02.123	ICU	680	+		
02.123	ICU	669	+		
02.123	ICU	676	+		
05.312	Maternity Ward	626	+	712	-
05.312	Maternity Ward	625	+	715	+
05.312	Maternity Ward	624	+	714	+
05.312	Maternity Ward	622	+	704	+
05.312	Maternity Ward	621	+	716	+
05.312	Maternity Ward	620	+	711	-
05.312	Maternity Ward	619	+	703	+
05.312	Maternity Ward	618	+	710	-

RoomNumber	SampleGroup	1-SampleID	1-16S	2-SampleID	2-16S
05.312	Maternity Ward	617	+	706	-
05.312	Maternity Ward	631	+	708	+
05.322	Maternity Ward	888	+	816	-
05.322	Maternity Ward	889	+	810	+
05.322	Maternity Ward	892	+	806	+
05.322	Maternity Ward	893	-	804	+
05.322	Maternity Ward	894	+	815	-
05.322	Maternity Ward	899	+	802	+
05.334	Maternity Ward	1096	+	1174	-
05.334	Maternity Ward	1093	+	1178	+
05.334	Maternity Ward	1099	+	1176	-
05.334	Maternity Ward	1098	+	1179	+
05.334	Maternity Ward	1102	+	1186	+
05.334	Maternity Ward	1100	+	1180	-
05.334	Maternity Ward	1092	+	1187	+
05.334	Maternity Ward	1088	+	1181	+
05.334	Maternity Ward	1086	+	1182	+
05.334	Maternity Ward	1097	+	1173	+
06.244	Pediatric Ward	430	+	639	-
06.244	Pediatric Ward	424	+	644	-

RoomNumber	SampleGroup	1-SampleID	1-16S	2-SampleID	2-16S
06.244	Pediatric Ward	427	+	645	-
06.244	Pediatric Ward	428	+	648	+
06.244	Pediatric Ward	426	+	638	-
06.244	Pediatric Ward	417	+	636	+
06.244	Pediatric Ward	418	+	633	+
06.244	Pediatric Ward	419	+	637	-
06.244	Pediatric Ward	420	+	643	+
06.244	Pediatric Ward	422	+	635	+
06.244	Pediatric Ward	425	+	646	-
06.244	Pediatric Ward	423	+	641	+
06.244	Pediatric Ward	431	+	634	+
06.244	Pediatric Ward	432	+		
06.244	Pediatric Ward	429	+	640	-
06.248	Pediatric Ward	448	-	558	+
06.248	Pediatric Ward	447	+	542	-
06.248	Pediatric Ward	445	+	476	+
06.248	Pediatric Ward	443	+	550	+
06.248	Pediatric Ward	434	+	513	-
06.248	Pediatric Ward	442	+	581	+
06.248	Pediatric Ward	433	+	361	+

RoomNumber	SampleGroup	1-SampleID	1-16S	2-SampleID	2-16S
06.248	Pediatric Ward	435	+	597	+
06.248	Pediatric Ward	436	+	490	+
06.248	Pediatric Ward	437	+	510	-
06.248	Pediatric Ward	438	+	354	+
06.248	Pediatric Ward	439	+	598	+
06.248	Pediatric Ward	440	+	357	+
06.248	Pediatric Ward	441	+	514	-
06.312	Surgical Ward	1083	+	1199	+
06.312	Surgical Ward	1070	+	1189	+
06.312	Surgical Ward	1075	-	1195	+
06.312	Surgical Ward	1076	+	1192	+
06.312	Surgical Ward	1078	+	1204	+
06.312	Surgical Ward	1079	+	1201	+
06.312	Surgical Ward	1080	-	1194	+
06.312	Surgical Ward	1081	+	1190	+
06.312	Surgical Ward	1082	-	1202	+
06.312	Surgical Ward	1085	-	1197	+
06.316	Surgical Ward	921	+	870	+
06.316	Surgical Ward	920	+	875	-
06.316	Surgical Ward	931	+	879	+
06.316	Surgical Ward	930	+	869	-
06.316	Surgical Ward	929	-	883	+
06.316	Surgical Ward	926	+	873	-
06.316	Surgical Ward	925	+	885	-
06.316	Surgical Ward	923	+	874	+
06.316	Surgical Ward	919	+	882	-
06.316	Surgical Ward	922	+	877	-
06.316	Surgical Ward	918	+	871	-
06.316	Surgical Ward	917	+	872	+
06.316	Surgical Ward	916	+	881	+
06.338	Surgical Ward	717	-	768	+
06.338	Surgical Ward	719	+	771	-
06.338	Surgical Ward	723	+	780	+
06.338	Surgical Ward	729	+	772	+

RoomNumber	SampleGroup	1-SampleID	1-16S	2-SampleID	2-16S
06.338	Surgical Ward	730	+	775	+
06.342	Surgical Ward	402	+	690	-
06.342	Surgical Ward	403	+	682	+
06.342	Surgical Ward	414	-	695	+
06.342	Surgical Ward	416	-	689	+
06.342	Surgical Ward	406	+	686	+
06.342	Surgical Ward	404	+	681	-
06.342	Surgical Ward	407	+	684	-
06.342	Surgical Ward	408	+	687	-
06.342	Surgical Ward	409	+	691	+
06.342	Surgical Ward	411	+	692	+
06.342	Surgical Ward	405	+	685	-
06.342	Surgical Ward	410	+	693	+
07.216	Medical Ward	1013	-	1153	+
07.216	Medical Ward	1012	+	1148	+
07.216	Medical Ward	1014	+	1144	+
07.216	Medical Ward	1002	+	1146	-
07.216	Medical Ward	1006	+	1147	+
07.216	Medical Ward	1011	+	1138	+
07.216	Medical Ward	1015	+	1141	-
07.216	Medical Ward	1005	+	1149	+
07.242	Medical Ward	1053	+	1039	+
07.242	Medical Ward	1065	-	1043	+
07.242	Medical Ward	1062	+	1045	+
07.242	Medical Ward	1059	+	1048	+
07.242	Medical Ward	1057	+	1049	+
07.242	Medical Ward	1055	-	1035	+
07.242	Medical Ward	1066	-	1042	+
07.242	Medical Ward	1056	+	1051	+
07.244	Medical Ward	830	+	855	+
07.244	Medical Ward	827	+	853	-
07.244	Medical Ward	831	+	858	+
07.244	Medical Ward	828	+	864	-
07.244	Medical Ward	825	-	852	+
07.244	Medical Ward	819	+	861	+
07.304	Medical Telemetry	475	+	787	+
07.304	Medical Telemetry	468	+	791	-

RoomNumber	SampleGroup	1-SampleID	1-16S	2-SampleID	2-16S
07.304	Medical Telemetry	469	+	790	-
07.304	Medical Telemetry	473	+	782	+
07.304	Medical Telemetry	465	+	795	+
07.304	Medical Telemetry	478	+	785	+
07.304	Medical Telemetry	479	+	788	-
07.304	Medical Telemetry	480	+	794	+
07.304	Medical Telemetry	467	+	792	-
07.304	Medical Telemetry	471	+	796	+
07.328	Medical Telemetry	485	+	747	-
07.328	Medical Telemetry	495	+	737	-
07.328	Medical Telemetry	494	+	739	+
07.328	Medical Telemetry	493	+	734	-
07.328	Medical Telemetry	492	+	744	-
07.328	Medical Telemetry	491	+	733	-
07.328	Medical Telemetry	489	+	735	-
07.328	Medical Telemetry	488	+	746	-
07.328	Medical Telemetry	486	+	742	+
07.328	Medical Telemetry	484	+	738	+
07.328	Medical Telemetry	487	+	740	-
07.334	Medical Telemetry	449	+	614	-

RoomNumber	SampleGroup	1-SampleID	1-16S	2-SampleID	2-16S
07.334	Medical Telemetry	452	+	602	-
07.334	Medical Telemetry	453	+	608	-
07.334	Medical Telemetry	455	+	613	-
07.334	Medical Telemetry	456	+	605	-
07.334	Medical Telemetry	457	+	611	-
07.334	Medical Telemetry	458	+	606	+
07.334	Medical Telemetry	459	+	610	-
07.334	Medical Telemetry	460	+	615	+
07.334	Medical Telemetry	461	+	612	-
07.334	Medical Telemetry	464	+	603	-
07.334	Medical Telemetry	463	+	604	-
07.336	Medical Telemetry	1103	+	1133	+
07.336	Medical Telemetry	1105	-	1130	+
07.336	Medical Telemetry	1106	+	1123	+
07.336	Medical Telemetry	1115	+	1127	+
07.336	Medical Telemetry	1113	+	1121	+
07.336	Medical Telemetry	1111	+	1137	-
07.336	Medical Telemetry	1109	-	1135	+
07.336	Medical Telemetry	1108	+	1125	-
07.336	Medical Telemetry	1104	-	1134	+

RoomNumber	SampleGroup	1-SampleID	1-16S	2-SampleID	2-16S
07.336	Medical Telemetry	1110	+	1120	+
07.342	Medical Telemetry	848	+	781	-
07.342	Medical Telemetry	847	+	901	-
07.342	Medical Telemetry	846	+	902	-
07.342	Medical Telemetry	844	+	903	-
07.342	Medical Telemetry	843	+	907	+
07.342	Medical Telemetry	839	+	905	+
07.342	Medical Telemetry	837	+	911	-
07.342	Medical Telemetry	841	+	912	+
07.342	Medical Telemetry	835	+	910	-
07.342	Medical Telemetry	836	+	914	+

BELVOIR Acinetobacter

RoomNumber	SampleGroup	Surface	1-SampleID	1-ACB	2-SampleID	2-ACB
06.342	Surgical Ward	Sink top	403	-	682	x
06.342	Surgical Ward	Call box	405	x	685	
06.342	Surgical Ward	Bedside table	406	x	686	-
06.342	Surgical Ward	Room door closer	407	x	684	
06.342	Surgical Ward	Toilet rail	409	-	691	+
06.342	Surgical Ward	COMPOSITE 1	410	-	693	+
06.342	Surgical Ward	Tray table	411	-	692	x
06.342	Surgical Ward	Toilet handle	414		695	x
06.342	Surgical Ward	COMPOSITE 2	416		689	+
06.244	Pediatric Ward	Tray table	422	-	635	x
06.248	Pediatric Ward	COMPOSITE 1	435	x	597	+
06.248	Pediatric Ward	Toilet rail	436	-	490	x
06.248	Pediatric Ward	Toilet seat	439	-	598	+
06.248	Pediatric Ward	Room door closer	445	-	476	+
07.334	Medical Telemetry	Call box	452	x	602	
07.334	Medical Telemetry	COMPOSITE 1	453	+	608	
07.334	Medical Telemetry	Sink top	459	x	610	
07.304	Medical Telemetry	Toilet seat	465	-	795	x
07.304	Medical Telemetry	PT chair	471	-	796	x
07.304	Medical Telemetry	Tray table	473	-	782	+
07.304	Medical Telemetry	Telephone	475	-	787	x
07.304	Medical Telemetry	COMPOSITE 2	478	x	785	x
07.304	Medical Telemetry	COMPOSITE 1	480	-	794	x

RoomNumber	SampleGroup	Surface	1-SampleID	1-ACB	2-SampleID	2-ACB
07.328	Medical Telemetry	Tray table	484	x	738	-
07.328	Medical Telemetry	Toilet seat	488	x	746	
05.312	Maternity Ward	COMPOSITE 1	619	-	703	+
05.312	Maternity Ward	Toilet handle	621	-	716	x
05.312	Maternity Ward	Toilet seat	624	-	714	x
05.312	Maternity Ward	COMPOSITE 2	625	-	715	x
05.312	Maternity Ward	PT chair	631	-	708	x
02.122	ICU	COMPOSITE 2	650	-	759	x
02.122	ICU	Tray table	656	-	762	+
02.122	ICU	COMPOSITE 1	661	-	750	+
02.123	ICU	COMPOSITE 1	680	x		
06.338	Surgical Ward	COMPOSITE 2	717		768	+
06.338	Surgical Ward	Sink top	719	x	771	
06.338	Surgical Ward	COMPOSITE 1	723	x	780	-
06.338	Surgical Ward	Tray table	729	-	772	x
06.338	Surgical Ward	Toilet rail	730	+	775	-
06.338	Surgical Ward	Toilet handle	731	+	779	
07.244	Medical Ward	Room sink	831	-	858	x
07.342	Medical Telemetry	Toilet handle	835	x	910	
07.342	Medical Telemetry	Sink top	836	x	914	-
07.342	Medical Telemetry	Toilet seat	837	x	911	
07.342	Medical Telemetry	Toilet rail	839	x	905	x
07.342	Medical Telemetry	Telephone	844	x	903	
07.342	Medical Telemetry	Room Sink	846	x	902	
07.342	Medical Telemetry	Chair	847	x	901	
05.322	Maternity Ward	Bedside table	892	-	806	x

RoomNumber	SampleGroup	Surface	1-SampleID	1-ACB	2-SampleID	2-ACB
05.322	Maternity Ward	IV Pole	893		804	x
06.316	Surgical Ward	Toilet rail	916	+	881	-
06.316	Surgical Ward	IV Pole	917	+	872	-
06.316	Surgical Ward	Bedside table	918	+	871	
06.316	Surgical Ward	Chair	919	+	882	
06.316	Surgical Ward	Tray table	921	+	870	-
06.316	Surgical Ward	Room Sink	923	+	874	+
06.316	Surgical Ward	Toilet seat	925	+	885	
06.316	Surgical Ward	Call box	926	+	873	

A Comparison of Culture and Real-Time PCR for Detecting Bacteria Directly from Environmental Swabs

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Running Title: Detection of nosocomial pathogens by RT-PCR

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ABSTRACT

Despite acknowledged limitations, swabbing with subsequent culturing remains the principal method for sampling environmental surfaces. Alternative, molecular techniques, such as real-time PCR, offer important advantages over culture dependent techniques, but concerns about specificity, accuracy and cost have hampered their widespread application.

318 environmental swabs were collected over a 5 month period from high touch surfaces in publicly accessible locations in three referral and teaching hospitals and 2 community hospitals in the National Capitol and Baltimore, Maryland regions. Swabs were plated onto Blood and Mac Conkey Agar plates and simultaneously tested for bacterial *16S* rRNA using real-time PCR. *16S* rRNA positive swabs were subsequently tested by real-time PCR for the presence of six clinically important nosocomial bacteria using a set of species-specific primers. Bacterial colonies from BAP and MAC plates were identified to the species level using the BD Phoenix Automated Microbiology System and compared to real-time PCR.

Bacterial growth and detection of *16S* rRNA were strongly correlated ($P<0.001$; Chi-squared). Four of the target organisms were isolated from 20 swabs using culture-based techniques, whereas 33 swabs, including all 20 culture-positive swabs, were positive by real-time PCR for five of target organisms, including *C. difficile*.

We demonstrate that real-time PCR can be successfully employed to detect clinically important bacteria from environmental swabs, and is comparable, and in some instances superior to culture-based techniques.

INTRODUCTION

Though initially believed to play an insignificant role (REF), environmental contamination is increasingly implicated in the transmission of hospital acquired infections (HAIs) (Otter 2011). However, ascertaining a definitive correlation between an environmentally contaminated surface and subsequent HAIs can be difficult, though studies have suggested that the extent of patient-to-patient transmission is directly proportional to the level of environmental contamination (REF). The inherent challenge in proving direct transmission via environmental contamination is further exacerbated by current methodologies for identifying and isolating bacteria species from surfaces. These techniques are essentially limited to culture-based methodologies, which suffer from multiple deficiencies, including labor- intensive culturing methods, complicated growth requirements for some bacterial species, and, in particular, the ability to separate bacterial species of interest from a mixed bacterial flora, particularly when the target organism is present in low concentrations.

In recent years, efforts to develop rapid and accurate molecular detection assays have been proposed (REF). Molecular methods can provide significant advantages over traditional techniques, including high sensitivity, rapid turnaround time, and greater flexibility in detecting new targets. However, no assay is perfect, and the limitations of molecular methods, including cross-reactivity with other species and a lack of molecularly trained technicians (REF, Maurer2011), have contributed to their sluggish implementation. This is particularly relevant when dealing with environmental surfaces, where multiple species of varying concentrations can be present (REF).

Bacterial *16S* rRNA is a desirable primary target, primarily due to its evolutionary conservation among disparate bacterial species, and has been used in multiple assays for detecting bacteria (REF). Its broad range makes it unsuitable for species identification, but other assays targeting clinically relevant bacteria have also been developed (REF). A number of these assays, most notably for the detection of

Methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococci* (VRE), and toxic *Clostridium difficile* have been translated into the clinical setting, usually via automated instrumentation, but they are costly to maintain and have very specific targets.

We recently developed and validated a set of species specific primers for MRSA (Mc Gann ICHE), *Acinetobacter baumannii, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa*, as well as a novel bacterial *16S* rRNA real-time PCR primer which will amplify a product from 93.8% of all bacterial *16S* rRNA sequences published to date (REF Clifford 2012). In this report, we take this assay into the field in an effort to directly compare culture and real-time PCR for the detection of bacterial pathogens of clinical importance directly from environmental swabs. The study was performed over a five-month period, using environmental swabs collected from high-tough surfaces in 5 major healthcare facilities in the Washington DC and Baltimore Area. Our data demonstrate that real-time PCR was not only directly comparable to culture-based techniques in detecting the presence of potentially pathogenic bacteria, but it was also capable of detecting organisms missed by culture-based techniques.

MATERIALS AND METHODS

Real-time PCR primers

All real-time PCR primers, with the exception of those targeting *C. difficile* have been described and validated previously (REF). The lack of complete genome sequences and the difficulty in isolating sufficient quantities of different strains of *C. difficile* for validation made design of a specific primer set challenging. Therefore, we used the primers targeting the toxin gene A (*tcdA*) designed by Knetsch and colleagues (REF). The primers were tested for specificity against a panel of American Type Culture Collection strains (ATCC, Manassas, VA) and clinical isolates representing fifty different bacterial species as described previously (REF). All reactions were performed in 20 µl volumes with 200 nM primers using a BioRad CFX96 with SsoAdvanced SYBR Green supermix (Bio-Rad Laboratories, Hercules, CA).

Optimization of swabbing protocol

Initial optimization studies were conducted under controlled conditions in the laboratory using spiked environmental surfaces. Serial dilutions of target organisms were spread evenly onto sterilized surfaces of defined sizes ranging from one square inch to four square inches and allowed to dry. A variety of swab types were tested for bacterial recovery, including standard sterile cotton swabs, pre-moistened sterile cotton swabs, large-sized 3M Sponge-Stick swabs (3M Center, St. Paul, MN), and liquid Amies Medium transport swabs (Copan Diagnostics Inc, Murrieta, CA) that support bacterial preservation during extended transport. Swabbing times were varied from 10 seconds to 30 seconds.

Isolation and identification of bacterial species by culture

Swabs were streaked directly onto Blood (BAP) and Mac Conkey (MAC) agar plates without any broth enrichment of the swabs (see Results) and incubated at 37^{o}C for 48 hours. All BAP and MAC plates were examined for growth by two microbiologists with clinical microbiology experience. Individual

colonies were isolated, purified and identified to the species level using the BD Phoenix Automated Microbiology System (Diagnostics Systems, Sparks, MD).

Identification of bacterial species by real-time PCR

Immediately after streaking, the swab heads were aseptically removed and added to 300 µl of either sterile phosphate buffered saline (PBS) or ultra-pure water (See Results) and vortexed vigorously for 1 minute. A 20 µl aliquot was removed and used directly for DNA preparation using Lyse-and-go as described previously (Mc Gann NDM plasmid). Briefly, the 20 µl aliquot was added to 20 µl of Lyse-and-go reagent (Thermo Scientific, Waltham, MA) in 96-well plates, and run in a thermal cycler using the manufacturer's protocol for the isolation of total genomic DNA. Isolates were held at 80^{o}C for 15 minutes at the end of the program to maximize lysis, and 2 µl of the resulting lysate was used directly for real-time PCR. Appropriate positive, negative, and no template controls (Ultra-pure water) were incorporated onto every plate. Cycling parameters were 95^{o}C for 5 minutes, followed by 40 cycles of 95^{o}C for 10 seconds and 56^{o} C for 10 seconds. A melting curve analysis was included at the end of every program to assist in data analysis. Quantification cycle (Cq) was calculated automatically using instrument software.

Collection and processing of environmental swabs

Environmental swabs were collected from July 2011 through November 2011 from a variety of publically accessible areas, such as restrooms and waiting areas, within healthcare centers in the National Capitol and Baltimore, MD regions. (supplemental Table SX). Based on CDC recommendations for environmental sampling, seventeen high-touch surfaces with corresponding clinical significance were selected, including door handles, light switches, bathroom rails, sink faucets, hand rests, and telephones. Where clinical surrogates were not readily obvious, surfaces with similar functions were selected instead, such as elevator buttons in place of call buttons (Supplemental Table

S1). Each swab collection protocol was conducted over two days, with the same seventeen sites swabbed on both days at different times of the day to account for cleaning schedules. Swabs were collected from each healthcare center on two separate collection periods over the 5 month period. Swabs were immediately transported to the research laboratory and streaked onto BAP and MAC plates, and tested for *16S* rRNA and the target organisms by real-time PCR as described above.

Sequencing of *16S rRNA* gene by PCR

Where appropriate, a 1,465bp sequence of *16S* rRNA was amplified by conventional PCR using a universal primer pair described by Weisburg and co-workers (Weisburg 1992). Amplicons were sequenced by Macrogen Corp (Rockville, MD, USA) and assembled using SeqMan and aligned using MegAlign (DNAStar Inc., Madison, WI, USA).

Statistical analyses

Where appropriate, statistical analyses were performed using Sigma Plot 12.0 (Systat Software, Inc, San Jose, CA).

RESULTS

Optimal swabbing parameters

Swab type, swabbing technique, and choice of diluents had significant effects on experimental outcome.

Both the pre-moistened and the Amies Medium transport swabs demonstrated comparable results in the recovery of bacteria from contaminated surfaces (data not shown), but the greater versatility in transport time offered by the Amies Medium transport swabs proved to be the deciding factor in choosing the optimal swab. As expected, dry cotton swabs were the least effective in collecting bacterial samples. Similarly, the sized 3M Sponge-Stick were too large, requiring submersion in over 20 mL of diluent to adequately cover the entire swabs, which resulted in a concomitant decrease in the sensitivity of real-time PCR to detect bacterial *16S rRNA* (data not shown).

Swabbing time was varied from 10 to 30 seconds, and initial optimization studies using spiked benches indicated that swabbing for 20 or 30 seconds consistently resulted in 4-8 fold higher yields of amplified product (as determined by Cq) compared to swabbing for just 10 seconds (Figure 1a). In an effort to reduce the number of swabs required for the study, single composite swabs were used to swab all surfaces both before and after individual swab use. However, culture data indicated that the composite swab was consistently less efficient at collecting a representative sample of the microbial flora when compared to individual swabs. Based on these results, use of the composite swab was discontinued prior to the commencement of the study.

Finally, detection of bacterial DNA was conducted from swabs immersed in both PBS and water. However, validation studies demonstrated that real-time PCR was adversely affected by the higher salt concentration in PBS, which resulted in a 2 to 3 log reduction in signal, even after the sample was diluted in the lyse-and-go buffer (Figure 1b).

The final swabbing protocol required firmly swabbing an area no less than 5 square centimeters for 20 seconds with pre-moistened cotton swabs from up to 17 high touch surfaces in publically accessible areas. Swabs were rolled during the 20 second sampling to ensure maximal contact area of the swab tip, streaked onto BAP and MAC plates, and incubated for up to 48 hours before colony isolation and subsequent identification. Immediately after streaking, swab heads were aseptically removed and added to 300 µl of sterile, ultra-pure water and vortexed vigorously for 45 seconds.

Optimized real-time PCR conditions for use with environmental swabs

All primer sets were capable of reproducibly detecting as few as 3×10^2 genome copies from purified genomic DNA (Clifford 2012). This capability was unaffected by the use of mixed bacterial cultures, including a cocktail prepared from the serial dilution of a mix of *A. baumannii*, *E. coli*, *Klebsiella pneumoniae*, and *P. aeruginosa*.

Despite the unavoidable dilution factor, real-time PCR consistently detected bacterial *16S rRNA* from spiked surfaces, even when bacterial load was as low as 1×10^3 organisms/mL. Consequently, all swabs were immersed in water after culturing and stored at -20^{o}C. Monthly, swab samples were removed, thawed and retested with both the *16S rRNA* primer set and the corresponding species-specific primer set where appropriate. No appreciable degradation in real-time PCR signal has been observed from any sample to date.

Bacterial contamination of environmental surfaces using culture-based techniques

Three hundred and eighteen swabs were collected over the five month sampling period, and growth on BAP was observed with 217 (68.2%). Individual colonies were selected from 89 swabs, including 5 swabs where >1 colonies were chosen. Ninety-five individual isolates, spanning 15 genera where identified (Table 2). Twenty isolates from the five target bacterial species were identified, with *A. baumannii/ A. baumannii/calcoaceticus* complex predominating (55%), followed by *P. aeruginosa*

(30%), *E. coli* (10%) and *K. pneumoniae* spp. *pneumoniae* (5%). Two isolates of *S. aureus* were also isolated, but were methicillin-sensitive by oxacillin screen (REF).

Bacterial contamination of environmental surfaces using real-time PCR

217 swabs were both culture and *16S rRNA* positive and bacterial growth was strongly concordant with *16S rRNA* gene detection ($P<0.001$; Chi-square with Yates correction). Growth on BAP or MAC without a corresponding detection of *16S rRNA* signal was observed from just five swabs (supplemental Table S1), and subsequent culture and microscopic analyses demonstrated that this growth was due to fungi and molds. A positive *16S rRNA* signal, without corresponding growth on either BAP or MAC, was detected from eight swabs. To determine whether this was due to the presence of viable, but non-culturable bacteria (REF), full *16S rRNA* sequencing was performed using the universal conventional PCR primer set designed by Wurziel and colleagues (REF). Three of the eight sequences could not be assembled as they consisted of overlapping *16S rRNA* sequences, indicating the presence of multiple bacterial species. The remaining amplicons had *16S rRNA* sequences that were 100% identical to *Pseudomonas putida* (4 swabs) and *Stenotrophomonas maltocida* (1 swab). As both these species can be isolated using the growth conditions employed in this study, the absence of a corresponding culture from these swabs could be the result of a number of factors, including very low numbers of bacteria in the swabs, non-viable bacterial cells, or technical errors.

Real-time PCR detected DNA from five of the six target organisms on 35 swabs, compared to just 20 from culture. *A. baumannii/ A. baumannii/calcoaceticus* complex was the most prevalent (42.4%), followed by *P. aeruginosa* (39%), *E. coli* (12.1%) and *K. pneumoniae* spp. *pneumoniae* (6%). *S. aureus* was detected on two swabs, but positive amplification was only detected from *femA*, and not with *mecA*, indicating the presence of methicillin-sensitive *S. aureus* (MSSA) (REF Mc Gann ICHE). Two of the swabs that were positive for *A. baumannii* were also positive for *C. difficile*, but due to the

fastidious growth requirements for this bacterium, these results could not be confirmed by culture-based methods. Crucially, every swab that was culture-positive for the target organisms was also positive for the same organism by real-time PCR, resulting in a 100% agreement between culture and real-time PCR detection methods. However, a further 13 swabs were positive by real-time PCR for one or more of the target organisms without a corresponding culture, including 7 *P. aeruginosa*, 3 *A. baumannii*, 2 *E. coli*, and 1 *K. pneumoniae* (Supplemental table X). These results were confirmed by melting curve analysis, which unambiguously detected amplicons with the same melting curve profile as the control strains.

DISCUSSION

Turnaround time from swab processing to results was less than 4 hours. This was greatly enhanced by first testing all swabs with a novel bacterial *16S* rRNA real-time primer, capable fo detecting >93% of all bacterial *16S* rRNA sequences published to date (Clifford 2012). A direct comparison between real-time PCR and culture-based techniques demonstrated almost 100% concordance, though real-time PCR did amplify products from 21 swabs without a corresponding culture.

To our knowledge, this is the first comprehensive report comparing culture and real-time PCR techniques in detecting bacterial contamination directly from environmental swabs, and demonstrates that real-time PCR has broad applicability with environmental surveillance.

As bacterial *16S rRNA* copy number can vary from just a single copy up to fifteen copies depending on the bacterial species (klappenbach 2001), the use of this target provides additional advantages in detecting the presence of bacteria.

application has been hampered by concerns about specificity (REF), and costs (REF).

Another limitation of our study is that we did not perform broth enrichment for MRSA.

Culture dependent methods for assessing the level of contamination of healthcare surfaces with MDRO are relatively slow and laborious. Culture based methods for detecting C. diff are especially laborious, and the current cost of molecular assays for C. diff make high volume screening of environmental surfaces difficult or impossible in cost-constrained settings.

Human error

A variety of factors can influence the efficacy and reproducibility of environmental swabbing, including choice of swab, swabbing time, swabbing technique, growth media for bacterial recovery, and the selection of appropriate target surfaces.

Detection of bacterial DNA from environmental swabs after using the swabs for direct culturing creates some technical challenges. Bacterial load would inevitably be lowered due to the culturing, and submersion of the swab heads in diluent would further dilute the number of available bacteria for detection.

melting curve analysis,

). For example, Huang and colleagues demonstrated that among patients whose prior room occupant was methicillin-resistant Staphylococcus aureus (MRSA) positive, a significantly higher number

(P=0.4) acquired MRSA compared to patients whose prior room occupant was MRSA-negative (REF). Similarly, Huang et al, and Nseir and colleagues have recently shown that admission to an ICU room previously occupied by a patient with c diff , or MDR *P. aeruginosa* or *A. baumannii* is an independent risk factor for acquisition of these bacteria by subsequent room occupants (REF).

REFERENCES

. Nadkarni MA, Martin FE, Jacques NA, Hunter N (2002) Determination of bacterial load by real-time PCR using a broad-range (universal) probe and primers set. Microbiology 148: 257-266.

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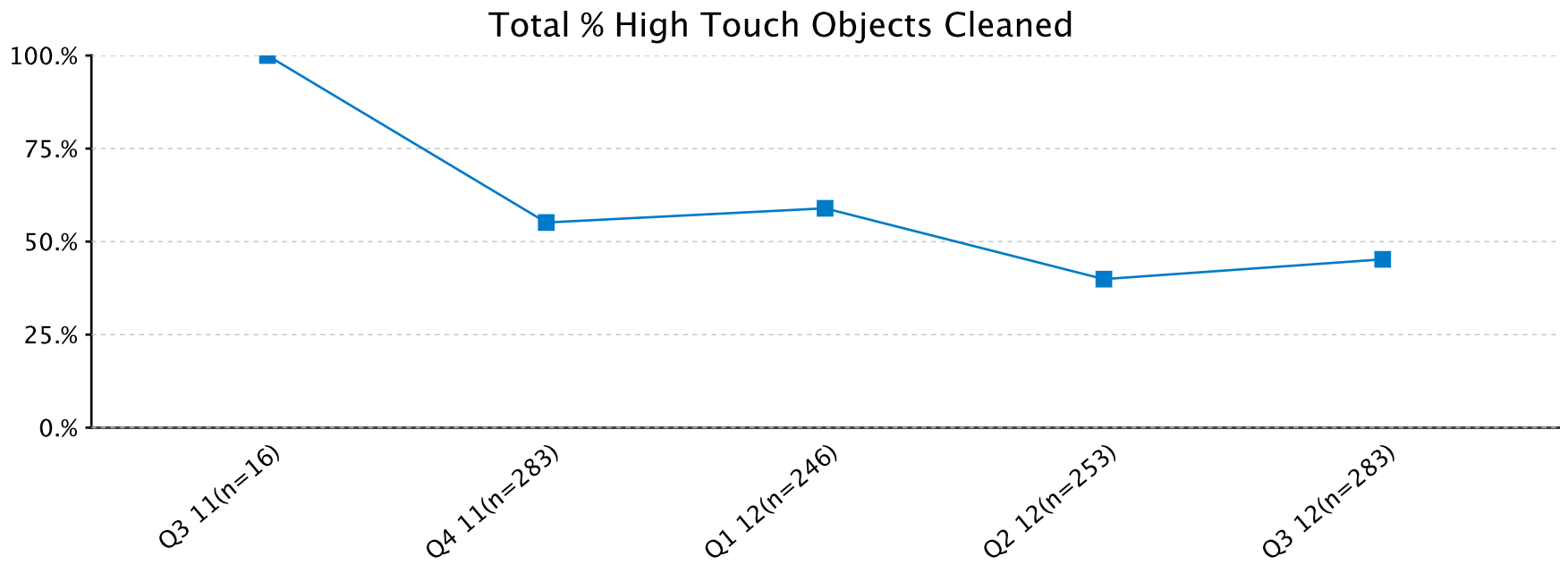
ENCOMPASS™ Environmental Hygiene Program

Dewitt Army Community Hospital

EnCompass™ Environmental Hygiene Results

ECOLAB

Total % High Touch Objects Cleaned



Include Baseline Results? yes, Dates between:2011-01-01 and 2012-09-26 Isolation Rooms?:All

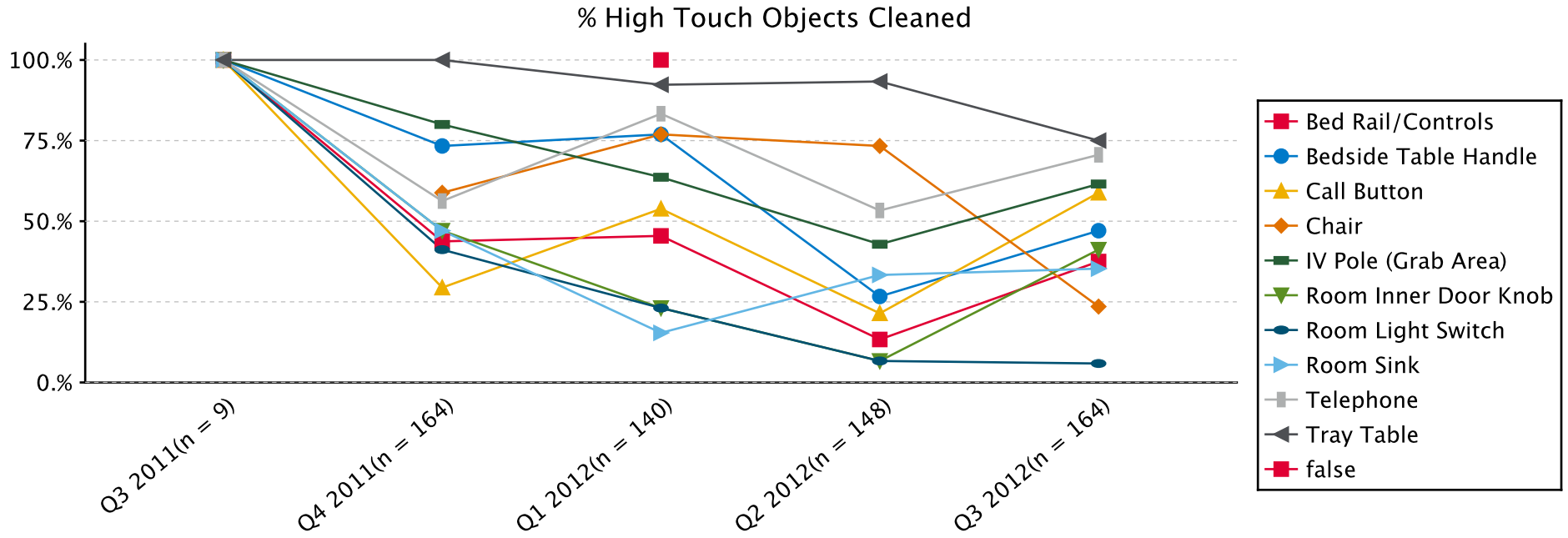
Focus Areas Report

Top 3 HTOs	% Cleaned
Toilet Seat	92 %
Tray Table	90 %
Telephone	66 %

Bottom 3 HTOs	% Cleaned
Room Light Switch	21 %
Bathroom Handrail by Toilet	22 %
Bathroom Light Switch	30 %

Include Baseline Results? yes, Dates between:2011-01-01 and 2012-09-26 Isolation Rooms?:All

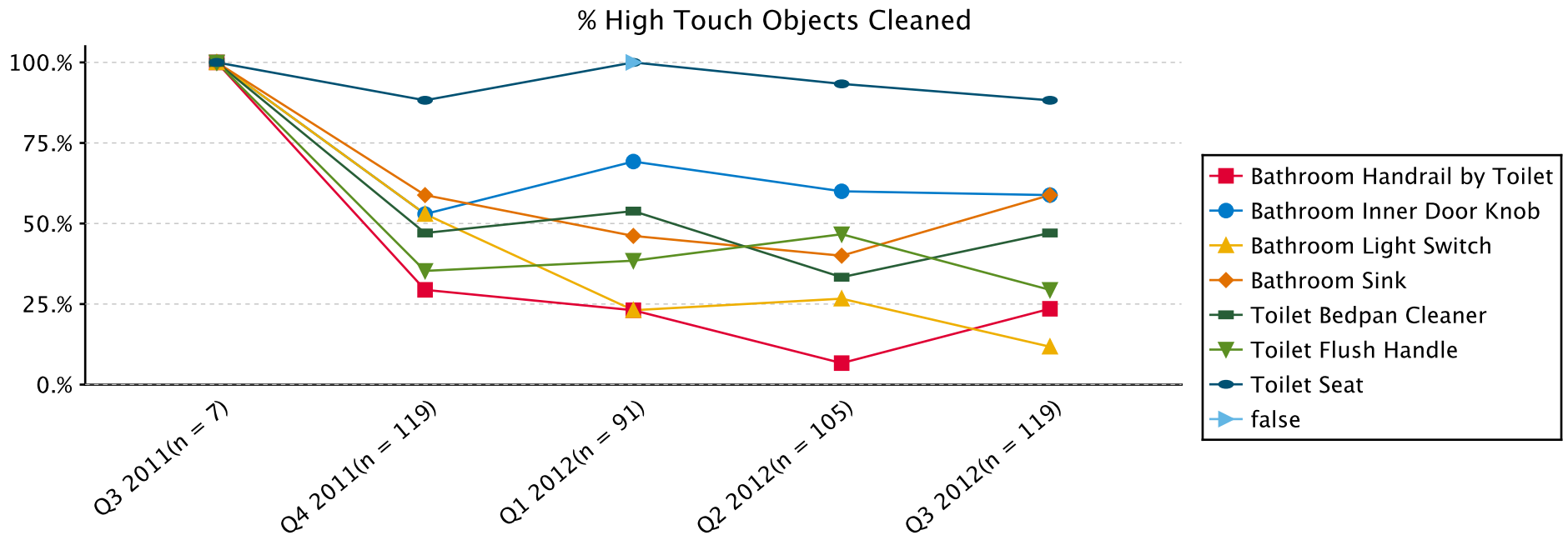
High Touch Object



Quarter	Bed Rail/Controls	Bedside Table Handle	Call Button	Chair	IV Pole (Grab Area)	Room Inner Door Knob	Room Light Switch	Room Sink	Telephone	Tray Table			
Q3 2011	100 %	100 %	100 %		100 %	100 %	100 %	100 %	100 %	100 %			
Q4 2011	44 %	73 %	29 %	59 %	80 %	47 %	41 %	47 %	56 %	100 %			
Q1 2012	45 %	77 %	54 %	77 %	64 %	23 %	23 %	15 %	83 %	92 %			
Q2 2012	13 %	27 %	21 %	73 %	43 %	7 %	7 %	33 %	53 %	93 %			

Include Baseline Results? yes, Dates between:2011-01-01 and 2012-09-26 Isolation Rooms?:All

Q3 2012	38 %	47 %	59 %	24 %	62 %	41 %	6 %	35 %	71 %	75 %			



Quarter	Bathroom Handrail by Toilet	Bathroom Inner Door Knob	Bathroom Light Switch	Bathroom Sink	Toilet Bedpan Cleaner	Toilet Flush Handle	Toilet Seat			
Q3 2011	100 %	100 %	100 %	100 %	100 %	100 %	100 %			
Q4 2011	29 %	53 %	53 %	59 %	47 %	35 %	88 %			
Q1 2012	23 %	69 %	23 %	46 %	54 %	38 %	100 %			

Include Baseline Results? yes, Dates between:2011-01-01 and 2012-09-26 Isolation Rooms?:All

Q2 2012	7 %	60 %	27 %	40 %	33 %	47 %	93 %			
Q3 2012	24 %	59 %	12 %	59 %	47 %	29 %	88 %			

Include Baseline Results? yes, Dates between:2011-01-01 and 2012-09-26 Isolation Rooms?:All

Percent High Touch Object Cleaned

High Touch Objects	Baseline	Q2 2012	Q3 2012	Net Improvement (Over Baseline)	Trend (Over Last Period)
Bathroom Handrail by Toilet		7 %	24 %	24 %	Up
Bathroom Inner Door Knob		60 %	59 %	59 %	Down
Bathroom Light Switch		27 %	12 %	12 %	Down
Bathroom Sink		40 %	59 %	59 %	Up
Bed Rail/Controls		13 %	38 %	38 %	Up
Bedside Table Handle		27 %	47 %	47 %	Up
Call Button		21 %	59 %	59 %	Up
Chair		73 %	24 %	24 %	Down
IV Pole (Grab Area)		43 %	62 %	62 %	Up
Room Inner Door Knob		7 %	41 %	41 %	Up
Room Light Switch		7 %	6 %	6 %	Down
Room Sink		33 %	35 %	35 %	Up
Telephone		53 %	71 %	71 %	Up
Toilet Bedpan Cleaner		33 %	47 %	47 %	Up
Toilet Flush Handle		47 %	29 %	29 %	Down
Toilet Seat		93 %	88 %	88 %	Down
Tray Table		93 %	75 %	75 %	Down
Total Patient Bathroom		44 %	45 %	45 %	Up
Total Patient Room		37 %	45 %	45 %	Up
Grand Total		40 %	45 %	45 %	Up
n=(# of objects evaluated)		253	283		

< 70%
70% - 80%
> 80%

Include Baseline Results? yes, Dates between:2011-01-01 and 2012-09-26 Isolation Rooms?:All